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Fax Notes:

This is proposed examiner's amendment for 08/477,097. Please note that claim 109 needs to be canceled because it has the same scope as 112.

My telephone number is 571 272 0833. Anne Holleran

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EXHIBIT B
Applicants: Livingston et al.
U.S. Serial No.: 08/475,784
Filed: June 7, 1995

Application/Control Number: 08/477,097
Art Unit: 1642

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with xxx on xxx.

The application has been amended as follows:

In the specification:

at page 38, line 13, after "(Kensil et al. 1991)", the following was added:

Coursely chopped Q. saponaria bark [approximately 1 cm square, obtained from Hauser Chemicals, Boulder, CO] was stirred with 10 ml of water/g of bark at room termperature for 1 h. The extract was centrifuged and the supernatant containing the solubilized saponins was saved. The extraction step was repeated on the bark pellet and the two supernatants were pooled. To remove nonsaponin components, the supernatant pool was lyophilized, redissolved in 40 mM acetic acid in water at a concentration of 250 mg/ml (w:v) and either chromatographed through Sephadex G-50 (medium, Pharmacia, Piscataway, NJ) in 40 mM acetic acid with the hemolytic activity localized in the void volume fraction, or dialyzed against 40 mM acetic acid with the hemolytic activity retained by the dialysis membrane. The hemolytic fraction was lyophilized and redissolved at a concentration of 200 mg/ml in 40 mM acetic acid in

chloroform/methanol/water (62/32/6, v/v/v): 1 g of this fraction was applied to Silica Lichroprep (E.M. Science, Gibbston, NJ: 40 to 63 µm particle size, 2.5 cm I.D. x 20 cm height) and eluted isocratically in the solvent used to solubilize the saponins. The elution of saponins was monitored by carbohydrate assay. Fractions containing he saponins of interest were identified by reverse phase TLC with visualization with Bial's reagent (Sigma, ST. Louis, M)) pooled individually, and rotavapped to dryness. The fractions from the silica chromatography were then redissolved in 40 mM acetic acid in 50% methanol and loaded on a semipreparative HPLC column (Vydac C_4, 5µm particle size, 3000 nm pore size, 10 mm I.D. X 25 cm length). Saponin peaks detectd by absorbance at 214 nm were eluted by using a methanol gradient at a flow rate of 4 ml/min and individually rotavapped to dryness. Purity of saponins was assessed by analytic HPLC (Vydac C_4, 5µ particle size, 3000 nm pore size, 4.6 mm I.D. x 25 cm length) with a gradient of 0.1% TFA in acetonitrile. QS-21 is defined as the adjvant active reverse phase HPLC fraction 21 from Q. Saponaria bark extract.

In the claims:

Claim 109 was canceled.

Claim 100. A composition which comprises:

a) a conjugate of (i) a GM2 or a GD2 ganglioside derivative [which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base], wherein the GM2 or GD2 ganglioside derivative is a GM2 or GD2 ganglioside cleaved with ozone, and wherein an aldehyde group is introduced at

the C4 position of the sphingosine portion of the GM2 or GD2 ganglioside, and (ii) Keyhole Limpet Hemocyanin[;], wherein the GM2 or GD2 ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the sphingosine base and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin;

b) QS-21[, a saponin derivable from the bark of a Quillaja saponaria Molina tree]; and

c) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GM2 or GD2 ganglioside derivative is an amount between about 1 μg and about 200 μg, the amount of [the saponin] QS-21 is an amount between about 10 μg and about 200 μg, and the GM2 or GD2: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and [such saponin] QS-21 being effective to stimulate or enhance production in a subject of an antibody to GM2 and GD2, which ever is present as a derivative in the conjugate[,]

[wherein in the conjugate the ganglioside derivative is covalently bound to the derivative of Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin, wherein the C-4 carbon is present in a CH_2 group].

Claim 110. The composition of 100 wherein the amount of the [saponin] QS-21 is about 200 μg.

Claim 112 The composition of claim 100 which comprises:

a) a conjugate of (i) a GM2 or a GD2 ganglioside derivative [which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base], wherein the GM2 or GD2 ganglioside derivative is a GM2 or GD2 ganglioside cleaved with ozone, and wherein an aldehyde group is introduced at the C4 position of the sphingosine portion of the GM2 or GD2 ganglioside, and (ii) Keyhole Limpet Hemocyanin[;], wherein the GM2 or GD2 ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the sphingosine base and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin;

b) QS-21[, a saponin derivable from the bark of a Quillaja saponaria Molina tree]; and

c) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GM2 or GD2 ganglioside derivative is an amount between about 1 μg and about 200 μg, the amount of [the saponin] QS-21 is about 100 μg and the GM2 or GD2: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and [such saponin] QS-21 is effective to stimulate or enhance production in a subject of an antibody to GM2 and GD2, which ever is present as a derivative in the conjugate[,]

[wherein in the conjugate the ganglioside derivative is covalently bound to the derivative of Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin, wherein the C-4 carbon is present in a CH_2 group].

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Claim 114. A method of stimulating or enhancing production of an antibody directed to GM2 or GD2 in a subject which comprises administering to the subject an effective amount of a composition which comprises:

a) a conjugate of (i) a GM2 or a GD2 ganglioside derivative [which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base], wherein the GM2 or GD2 ganglioside derivative is a GM2 or GD2 ganglioside cleaved with ozone, and wherein an aldehyde group is introduced at the C4 position of the sphingosine portion of the GM2 or GD2 ganglioside, and (ii) Keyhole Limpet Hemocyanin[;], wherein the GM2 or GD2 ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the sphingosine base and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin;

b) QS-21[, a saponin derivable from the bark of a Quillaja saponaria Molina tree]; and

c) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GM2 or GD2 ganglioside derivative is an amount between about 1 μg and about 200 μg, the amount of [the saponin] QS-21 is an amount between about 10 μg and about 200 μg and the GM2 or GD2: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and [such saponin] QS-21 is effective to stimulate or enhance production in a subject of an antibody to GM2 and GD2, which ever is present as a derivative in the conjugate[,

wherein in the conjugate the ganglioside derivative is covalently bound to the derivative of Keyhole Limpet Hemocyanin by a stable amine bond

between the C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin, wherein the C-4 carbon is present in a CH_2 group], so as to thereby stimulate or enhance production of [the antibody] antibodies to GM2 and GD2 in the subject, whichever is present as a derivative in the conjugate.

Claim 115. A method of treating a human subject having cancer which comprises administering to the subject an effective cancer-treating amount of a composition which comprises:

a) a conjugate of (i) a GM2 or a GD2 ganglioside derivative [which comprises an unaltered oligosaccharide part and an altered ceramide portion comprising an altered sphingosine base], wherein the GM2 or GD2 ganglioside derivative is a GM2 or GD2 ganglioside cleaved with ozone, and wherein an aldehyde group is introduced at the C4 position of the sphingosine portion of the GM2 or GD2 ganglioside, and (ii) Keyhole Limpet Hemocyanin[;], wherein the GM2 or GD2 ganglioside derivative is covalently bound to the Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the sphingosine base and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin;

b) QS-21[, a saponin derivable from the bark of a Quillaja saponaria Molina tree]; and

c) a pharmaceutically acceptable carrier;

wherein the amount of the conjugated GM2 or GD2 ganglioside derivative is an amount between about 1 μg and about 200 μg, the amount of [the

saponin] QS-21 is an amount between about 10 μg and about 200 μg and the GM2 or GD2: Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1, the relative amounts of such conjugate and [such saponin] QS-21 is effective to stimulate or enhance production in a subject of an antibody to GM2 and GD2, which ever is present as a derivative in the conjugate], wherein in the conjugate the ganglioside derivative is covalently bound to the derivative of Keyhole Limpet Hemocyanin by a stable amine bond between the C-4 carbon of the altered sphingosine base of the altered ceramide portion of the ganglioside derivative and the nitrogen of the ε-aminolysyl group of Keyhole Limpet Hemocyanin, wherein the C-4 carbon is present in a CH_2 group], so as to thereby stimulate or enhance production of [the antibody] antibodies to GM2 and GD2 in the subject, whichever is present as a derivative in the conjugate.

Claim 125. The method of claim 124, wherein the conjugate and the [saponin] QS-21 are mixed on the day of administration to the subject.